Syringaldazine Oxidase and Phenylalanine Ammonia Lyase Activities in Relation to Lignin Deposition in Legumes

J.E. Bidlack, D.R. Buxton, R.M. Shibles and I.C. Anderson

Introduction

Increased knowledge of the temporal relationship between enzyme activity and cell-wall deposition will improve our understanding of wall biosynthesis and structure and help provide a foundation for targeted manipulation to improve wall digestibility. Among cell-wall components, lignin deserves attention because it provides plants with mechanical strength, protection against pests, and is thought to provide resistance to digestion by ruminant animals. Lignin deposition in the wall occurs during its thickening after the cell has completed elongation. Phenylalanine ammonia lyase (PAL), a general phenylpropanoid enzyme, and syringaldazine oxidase (SAO), a specific peroxidase, catalyze the first and last steps of the lignin biosynthetic pathway, respectively. The objectives of this investigation were to: 1) determine the temporal relationships of lignin deposition and PAL activity in relation to SAO activity, and 2) determine if SAO activity is more closely related to lignin content than PAL activity.

Materials and Methods

'Arrow' alfalfa, 'Viking' birdsfoot trefoil, and 'Arlington' red clover were grown in a greenhouse in 3.8 L pots with four replicates. The basal 10 cm of forage stems were sampled at 14, 28, 42, 56, and 70 days during regrowth. Acetone powders were prepared and analyzed for PAL and SAO activities. Cell-wall components, including lignin, were also determined. Enzyme activity was calculated on both a protein and a plant basis. Concentrations of cell-wall components over time were fitted with the Gompertz function and enzyme activities were fitted with a third-order quadratic equation. Times of maximum deposition were determined by setting the second derivative of the Gompertz function equal to 0 and solving for t (time). Times of maximum PAL and SAO activities were determined by setting the first derivative of the quadratic equal to 0 and solving for t.

Results and Discussion

Activities of PAL initially increased followed by decreased or plateaued activity as a function of regrowth days (Fig. 1). Significant decreases after peak activity of PAL were more apparent than the slight decreases of SAO activity after 25 days of growth. Neither PAL nor SAO activity on a protein basis were correlated with lignin content; however, PAL on a per plant basis was correlated with lignin content across species and SAO on a per plant basis was correlated with lignin content within species. Alfalfa, which consistently had the highest lignin content throughout the regrowth period, also exhibited higher wall-component depositions compared with the other species. Activity of PAL was also higher in alfalfa, but SAO activity of birdsfoot trefoil was at least three-fold higher than that of alfalfa throughout the growth period.

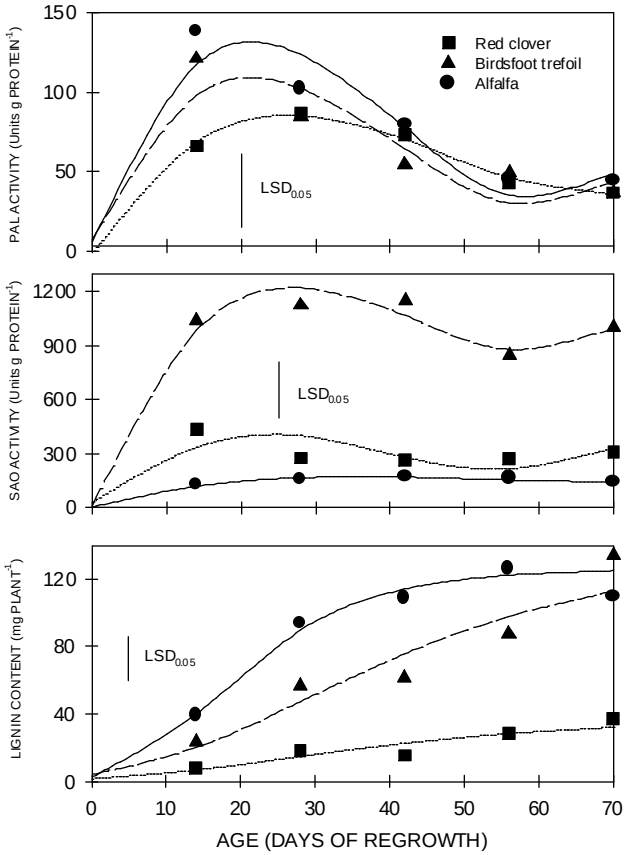

Figure 1. PAL and SAO activities and lignin content of basal stems of three legumes.

Time and extent of maximum lignin deposition in red clover lagged behind maximum deposition in alfalfa and birdsfoot trefoil (Table 1). Among cell-wall components, maximum hemicellulose deposition occurred first, followed by cellulose (1 to 3 days later) and then lignin (up to 14 days after maximum hemicellulose deposition). Maximum PAL activity preceded maximum SAO activity by 2 to 12 days.

Conclusions

Both PAL and SAO are related to lignin deposition on a per plant basis with maximum activity occurring first in PAL and then in SAO several days later.

Table 1. Time of maximum cell-wall component deposition on a per-plant basis and maximum PAL and SAO activities on a per protein basis in stems of three forage legumes.

	Species		
Measurement	Alfalfa	Birdsfoot trefoil	Red clover
	- - - - - - - - - days - - - - - - - - -		
Hemicellulose	13.0	25.5	20.6
Cellulose	14.7	28.7	23.9
PAL activity	20.3	21.5	25.3
SAO activity	32.1	26.4	27.3
Lignin	17.4	30.0	34.0